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***RESEARCH ARTICLE*.....**

Influence of chemical preservatives on the quality and shelf-life of dried Bombay duck (*Harpodon nehereus*)

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ABSTRACT..... Dry fish is the low cost dietary protein source in India. Sometimes dry fishes are kept for a longer period that is the key factor of the deterioration of nutritional value of dry fishes, as they absorb moisture from the surrounding air. In the present study Bombay duck (*Harpodon nehereus*) were treated separately with saturated brine (Control, C), dry salt with 0.25 per cent potassium sorbate (T_1) and saturated brine with 0.3 per cent sodium benzoate (T_2) and kept at ambient temperature for storage study after drying in open sun. The general purpose of this study is to determine the proximate composition of dry fishes treated with chemical preservatives and to investigate the quality changes of dried fishes with the increasing of storing period. The values for protein, fat, ash and moisture of dry fish for three different treatments were found in a range of 53.91 to 56.33 per cent, 5.94 to 11.77per cent, 14.78 to 21.96 per cent and 6.9 to 7.42 per cent, respectively. The results of biochemical parameters revealed that during storage period, TVBN, TMA and PV content of T_2 was better than C and T_1 whereas TBA of C was better than T_1 and T_2. The microbial parameters revealed that the quality of the dry fishes of both the samples (T_1 and T_2), treated with preservatives, were of good quality and remain acceptable for longer duration than the control (C). Depending upon the sensory evaluation the overall acceptability of the dried fishes (T_1, T_2 and C) was significantly declined during storage. The findings of this study showed that nutritional value of dry fishes treated with chemical preservatives give a better storage life than control.

KEY WORDS..... Drying, Bombay duck, Proximate composition, Biochemical parameter, Sensory evaluation

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INTRODUCTION.....

Fish is one of the major sources of proteins, vitamins and minerals and it has essential nutrients required for supplementing both infants and adults diet (Abdullahi *et al.*, 2001). Fish is an extremely perishable food item due to high content of moisture and soon after death it begins to spoil (Agbon *et al.*, 2002). According to Lilabati *et al.* (1999), there is a direct relationship between the microbial

count and moisture content of fish sample. It is necessary to reduce the moisture content of the fish as quickly as possible from (45 to 60 %) moisture content to 10 per cent moisture or less (Ruiter, 1995).

Although drying is regarded as a traditional, even primitive method of fish preservation in many developed countries, it still has an importance in the less developed regions of the world, and will remain so for a long time to come (Immaculate *et al*., 2013). Salting of fish followed by drying is a simple processing technique and it yields a product with relatively long shelf-life (Prakash *et al.,* 2011).

In last few years, there has been no steady result in the export of cured sea food, but quantity of processed sea food is substantial and cured products still occupy an important position in the sea food export business (MPEDA, 2013). Certain sections of the consumers of the country have developed a preference for such products because of its characteristic taste and flavour. In addition, these products cater to the need of weaker sections of Indian people living in the inland areas including plantation labours and hill people (Sen, 2005).

During the financial year 2012-13, export of marine products from India reached an all-time high of Rs.18,856 crores. The dried items exported from India was to the tune of 53,721 tonnes in the year 2011-12, which has increased to 72,953 tonnes in the year 2013-14 showing a positive growth in quantity of 35.80 per cent. In terms of value which was Rs.562.63 crores in the year 2011-12, has increased to Rs.819.90 crores (2013-14) with an increase of 45.72 per cent (MPEDA, 2013). The nutritional quality of dried fish remains intact, sometimes retains higher quality standards compared to fresh fish (Faruque *et al*., 2012).

Only in recent, there is a realisation that the market for dried product may be expanded by including economically better of sections who are ready to pay for better quality. With the available information it is known about the poor condition/quality of the product and therefore it was felt necessary to improve the quality by adding certain chemical preservatives which would help in providing better quality and also extending the shelf- life (Sen, 2005). The objectives of this study are to determine the proximate composition of dry fishes and to investigate the nutritional changes of the dry fishes treated with different chemicals during different storage.

RESEARCH METHODS.....

The fish sample Bombay duck (*Harpodon nehereus*) was collected in fresh condition from the landing centre Kakdwip, West Bengal, India and immediately brought them to the laboratory in iced condition in insulated boxes.

Proximate composition :

The proximate compositions of fresh and dried Bombay duck were determined following the standard method (AOAC, 1995). For moisture content determination, the samples were heated overnight in an electric oven at 60^0C. For ash content, ground dried samples were heated for 5 hrs in an electric oven 525^0C. Total protein content was calculated my multiplying kjeldahl nitrogen by 6.25.

Quality parameters :

For the determination of the quality of fresh and dried sample biochemical parameters like TMA (Tri-methyl amine), TVBN (Total volatile base nitrogen), PV (Peroxide Value) and TBARS (Thiobarbituric acid reactive substances) were observed. TVBN and TMA were estimated by using the method given by Conway (1947). The PV was determined as described by Jacobs (1958) and TBARS estimation was done by using a scientific kit of make Hi-Media CCK023-1000.

Microbiological characteristics:

Microbiological characteristics TPC (Total Plate Count) and TFC (Total Fungal Count) of fresh fish and dried fish were carried out. TPC was carried out as per the Standard methods (APHA, 1984). Like-wise mould count was enumerated on potato dextrose agar (PDA, 79).

Preparation of fish for drying :

Fishes were overnight treated with dry salt (NaCl) @ 0.8 kg salt/kg fish and 0.25 per cent potassium sorbate (weight basis) which was considered as treatment 1 (T_1). Another way dip treatment was done by overnight treatment with standard brine (Nacl @ 400 g salt in 1L of water) and 0.3 per cent sodium benzoate (weight basis) and considered as treatment 2 (T_2). Fishes treated overnight only with saturated brine considered as control (C).

Drying of fish :

The fishes were dried by following the drying practised in khotis of West Bengal, India. A bamboo structure was made in open sun and the fishes were kept hanging using hooks from the platform. During night time, the fishes were covered with polythene sheet in order to avoid uptake of moisture.

Packing :

After drying, the fishes were packed treatment-wise and also species wise in different low density polyethylene (LDPE) bags and tagged properly. Then these plastic bags were heat sealed and stored at room temperature for storage study.

Sensory analysis :

The quality determination of the products was made by trained panel of six (6) judges following 9-point hedonic scales (Peryan and Pilgrim, 1957). Comparison was carried out in terms of sensory characteristics such as odour, texture, and fish flavour intensity and general appearance. The panel was requested to rate each sensory feature of the salted product. The average score of 5 was considered to be the borderline of acceptability.

9-point	Hedonic scale
9	Very much extremely
8	Like extremely
7	Like
6	Like moderately
5	Like slightly
4	Dislike slightly
3	Dislike moderately
2	Dislike
1	Extremely dislike

Statistical analyses :

Data generated from the experiment were subjected to one way of analysis of variance using the SPSS (Statistical Package Computer, Software 1988 version Chicago Illinois, USA).

RESEARCH FINDINGS AND ANALYSIS.....

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Proximate composition of fresh fish :

The proximate composition of fresh Bombay duck (*Harpodon nehereus*) is given in (Table 1) which corroborates with the findings of Kumar *et al.* (2012).

Quality parameters of fresh fish :

For determination of the freshness of fish mainly TVBN, TMA and PV, TBARS was estimated. Sernapesca (2001) had reported that the maximum permitted level of volatile amines are 15 mg TMA/100g fish and 30 mg TVBN/100g fish for raw fish. In the present experiment, the values of TMA and TVBN of the specie (Table 2) were within the range which indicates that the raw fishes used were of good quality. And also the PV and TBARS value of fresh Bombay duck is presented in Table 2 which was indicating that fish was as fresh condition according to Immaculate *et al*. (2013) suggestion.

Microbial characteristics of fresh fish :

Surendran *et al*.(2006) mentioned that the acceptable limit of TPC for micro-organisms per g weight of fresh fish is 5 x10^5/g at 37°C. In the present study, the TPC values of fresh Bombay duck was found 2.18 x 10^4 CFU/g (Table 3) and no fungal growth which is indicating that the samples were in good quality.

Table 1 : Proximate composition of fresh fish

Species	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
Bombay duck	8.03 ± 1.09	2.01 ± 1.09	2.59 ± 1.27	86.72 ± 1.92

© Results are mean of three (n=3) determinations with s.d.

Table 2 : Quality parameters of fresh fish

Species	TMA (mg %)	TVBN (mg %)	PV (mili-equi O_2)	TBA (mg MDA/kg fat)
Bombay duck	3.79 ± 1.55	15.31 ± 1.4	1.69 ± 1.39	0

© Results are mean of three (n=3) determinations with s.d.

Drying of fish :

During the drying process, a significant reduction ($p< 0.05$) in moisture content was observed in all the treatments including control over a period of 10 days (Table 4). The minimum moisture content (6.9%) was observed in T_2 sample of Bombay duck showing 80 per cent reduction of moisture. Hoque *et al.* (2013) have reported that no microbe (yeast, mould and bacteria) can grow in a product with moisture content below 15 per cent. Analysis of variance among the different treatments reveals no significant ($p>0.05$) influence of potassium sorbate and sodium benzoate on drying process in relation to moisture content.

Proximate composition of the dried fishes during storage :

The experimental results show no such difference on the proximate composition of Bombay duck between two different treated samples (T_1 and T_2) and the control (Fig.1). Siddique and Aktar (2011) have reported similar trend of crude protein (58.33 %±0.4) for freshly dried *Harpodon nehereus*. As consider to fat the experimental result shows that the fat content of freshly dried Bombay duck ranges from 5.94 to 11.77 per cent. The mean values of ash content for dry fish of different treatments were observed 14.78 to 21.96 per cent. This result is supported by the result of Haque *et al.* (2013). The percentage of moisture found in dry Bombay duck of different treatments varied from 6.9 per cent to 7.42 per cent

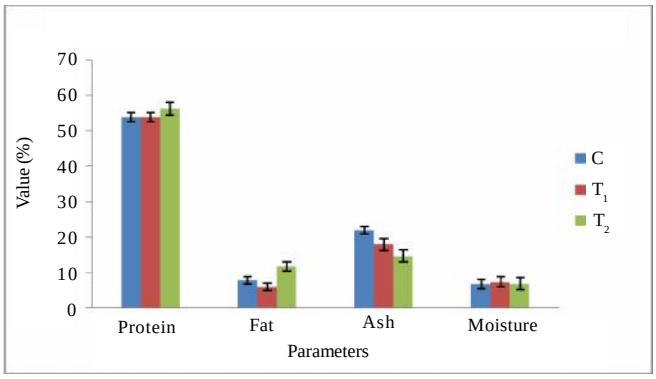

Fig. 1 : Proximate composition of dry Bombay duck of different treatments

Table 3 : Microbial characteristics of fresh fish

Parameters	Amount
TPC (cfu/g)	2.18×10^4
TPC (log cfu/g)	4.338
TFC (cfu/g)	0
TFC (log cfu/g)	0

Table 4 : Moisture content of Bombay duck (*Harpodon nehereus*) during drying

Days	Saturated brine (C) (%)	Dry salt and 0.25% potassium sorbate (T_1) (%)	Saturated brine and 0.3% sodium benzoate (T_2) (%)
0	86.72±1.1	86.72±1.21	86.72± 1.4
3rd	63.41±0.91	56.17±1.07	64.14±1.7
5th	49.60± 1.03	35.79±1.04	47.42±1.4
7th	22.59±0.96	25.14±0.97	32.32± 1.4
10th	6.91±0.71	7.42 ± 0.8	6.9 ±1.33

*Results are mean of three (n=3) determinations with sd.
Values of moisture content vary significantly ($p< 0.05$) within drying period and insignificantly ($p> 0.05$) between the treatment for a particular species

Table 5 : Changes of TVBN (mg %) value in dry Bombay duck during storage period

Days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T_1)	Saturated brine and 0.3% sodium benzoate (T_2)
0th	24.79 ± 1.6^{ab}	26.09 ± 1.07^{abc}	19.67 ± 1.07^{a}
30th	30.53 ± 2.4^{bcd}	28.09 ± 1.9^{abcd}	24.77 ± 1.21^{ab}
60th	35.91 ± 1.83^{cde}	32.60 ± 1.1^{bcde}	27.89 ± 1.26^{abcd}
90th	37.18 ± 1.17^{de}	36.46 ± 1.1^{de}	29.72 ± 1.71^{bcd}
120th	41.18 ± 1.7^{ef}	48.87 ± 1.6^{f}	29.94 ± 1.69^{bcd}

© *Results are mean of three (n=3) determinations with s.d. # Values of TVBN vary significantly ($p< 0.05$) between the treatment and storage period
@ Values with different superscript letter are significantly different within a row ($p< 0.05$).

(Fig. 1). The dried fish with 25 per cent or more moisture is not sufficient to inhibit microbial growth whereas dried fish with 15 per cent or less moisture is good enough to inhibit microbial growth. Similar cut-off point was also reported by Haque *et al.* (2013).

Changes in quality parameters of dried fishes during storage :

Storage study was done of samples (*Harpodon nehereus*) treated with different preservatives to assess the changes in quality of the products during its storage period. The TVBN values of T_1, C and T_2 samples were increased during storage period (Table 5) which is in concurrence with the result of Chakrobarti and Verma (2009) and Relekar *et al*. (2014). The TMA value increased in all the samples (Table 6) and such similar observation was also reported by Khuntia *et al.* (1990). During the storage period, the value for dry Bombay duck in T_1, C and T_2 samples increased significantly ($p< 0.05$).

PV was significantly ($p<0.05$) increased in all the dry samples during the storage which is supported by the findings of Kumar *et al.* (1997) (Table 7). Increase of peroxide value may be attributed to the oxidation of highly unsaturated fatty acids in fish lipids by the catalytic activity of common salt, pro-oxidant action of moisture and also auto oxidation by atmospheric oxygen (Priyadarshini *et al*., 2012).

TBA is widely used as an indicator for the assessment of degree of secondary lipid oxidation. TBA value of all the samples were increased significantly ($p<0.05$) during storage period (Table 8). At the end of storage period, the TBA values for dry Bombay duck of all treatments (T_1, C and T_2) were increased. However, the result shows significantly higher value ($p<0.05$) in T_1 and T_2 samples than the control one. It has been

Table 6 : Changes of TMA (mg %) value in dry Bombay duck during storage period

Days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T_1)	Saturated brine and 0.3% sodium benzoate (T_2)
0^{th}	11.89±1.87	10.4 ±1.9	11.03 ± 1.58
30^{th}	14.12±1.6	9.33 ± 1.31	11.26 ± 1.30
60^{th}	15.28±1.81	21.81±1.51	12.53±1.74
90^{th}	19.77±1.12	24.82 ± 1.4	14.88 ±1.37
120^{th}	19.53±1.12	24.82 ± 1.72	15.24 ± 1.73

© Results are mean of three (n=3) determinations with s.d.

Values of TMA content vary significantly ($p< 0.05$) within storage period and insignificantly ($p> 0.05$) between the treatments

Table 7 : Changes of peroxide value (mili-equi O_2 / kg fat) in dry Bombay duck during storage period

Days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T_1)	Saturated brine and 0.3% sodium benzoate (T_2)
0^{th}	12.22 ± 1.57^{ab}	6.88 ± 1.17^{a}	9.68 ± 1.1^{a}
30^{th}	15.45 ± 2^{ab}	13.37 ± 2.6^{ab}	10.71 ± 1.37^{ab}
60^{th}	15.62 ± 1.3^{abc}	23.64 ± 1.15^{bcd}	15.03 ± 1.47^{ab}
90^{th}	19.2 ± 1.01^{abcd}	29.14 ± 1.86^{cd}	17.21 ± 1.38^{abc}
120^{th}	32.14 ± 1.05^{d}	35.32 ± 1.09^{d}	16.98 ± 1.56^{abc}

©Results are mean of three (n=3) determinations with s.d. # Values of PV vary significantly ($p< 0.05$) between the treatment and storage period

@ Values with different superscript letter are significantly different within a row ($p< 0.05$).

Table 8 : Changes of TBA value (mg MDA / kg fish) in dry Bombay duck during storage period

Days	Saturated brine (Control) (C)		Dry salt and 0.25% potassium sorbate (T_1)		Saturated brine and 0.3% sodium benzoate (T_2)	
	µM MDA	mg MDA/kg	µM MDA	mg MDA/kg	µM MDA	mg MDA/kg
0^{th}	3.942±0.2	2.84±0.2	5.295±0.1	3.82±0.1	4.35±0.4	3.14±0.2
30^{th}	4.11±0.21	2.96±0.21	6.56±0.15	4.73±0.15	4.93±0.35	3.55±0.35
60^{th}	3.49±0.11	2.52±0.11	7.43±0.27	5.35±0.27	5.38±0.17	3.87±0.17
90^{th}	5.42±0.2	3.9±0.2	5.58±0.49	4.02±0.49	5.13±0.24	3.7±0.24
120^{th}	5.25±0.22	3.79±0.22	10.21±0.15	7.36±0.15	8.70±0.36	6.27±0.36

©Results are mean of three (n=3) determinations with s.d. # Values of TBA vary significantly ($p< 0.05$) between the treatment and storage period

@ Values with different superscript letter are significantly different within a row ($p< 0.05$)

suggested that a maximum TBA value indicating acceptable quality of the fish is 5 mg malonaldehyde/kg while fish may be consumed even up to 8 mg malonaldehyde (MA)/kg (Duman *et al.*, 2015).

Microbiological characteristics :

Throughout the period of storage TPC shows significant ($p< 0.05$) increasing trend in all the treatments (Fig. 2) after 120 days storage which is well corroborated with the findings of Oksüztepe and Yrfan (2010). Total fungal counts (TFC) were significantly ($p<0.05$) increased within a period of 120 days storage study among all the treatments (Fig.3). For dry fishes, the values were increased upto $5.4x10^6$ CFU/g (log value 6.64), $3.69x10^6$ CFU/g (log value 6.56) and $2.7x10^6$ CFU/g (log value 6.19) for C, T_1 and T_2 samples.

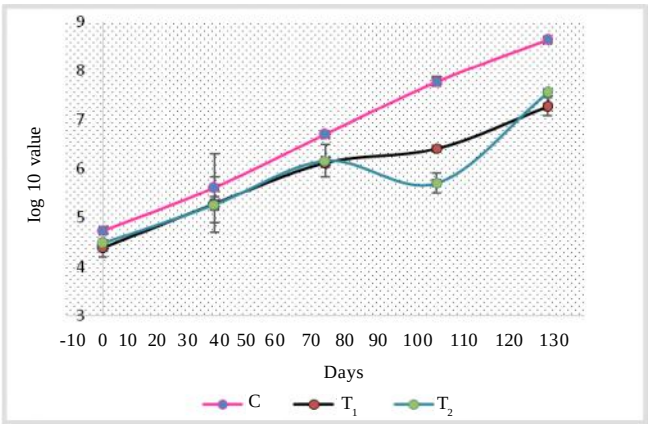

Fig. 2 : Changes of TPC log value in dry Bombay duck during storage

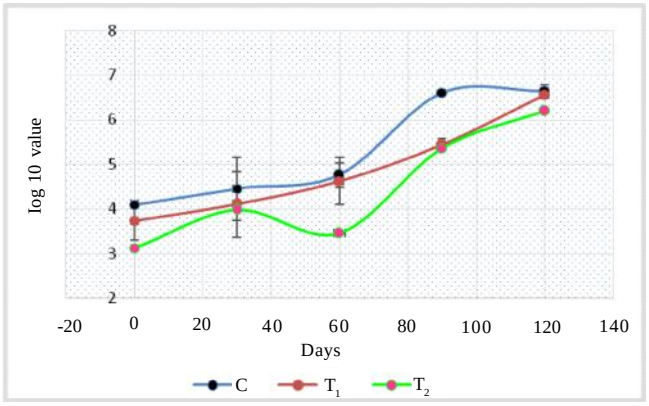

Fig. 3 : Changes of TFC log value in dry Bombay duck during storage

The rapid reduction in the water activity (a_w <0.75) is the most important factor in controlling fungi/mould contamination of the fishery products during storage (Kolakowska, 2002). But subsequent increase during storage period may be due to storage of the dry fishes at ambient temperature or may be due to absorption of moisture from atmosphere which accelerates the mould growth.

Sensory evaluation during storage :

The results of organoleptic evaluation or sensory ealuation during storage of dry Bombay duck of different samples (T_1, C, and T_2) shows that there was a decline in overall quality characteristics, namely colour and appearance, odour, texture, fish flavour intensity and overall acceptability during storage period of 120 days. The better quality of treated samples (T_1 and T_2) with preservatives (Fig.5 and 6) than the control samples (C) (Fig.4) was depicted by its higher scores throughout the storage period. The

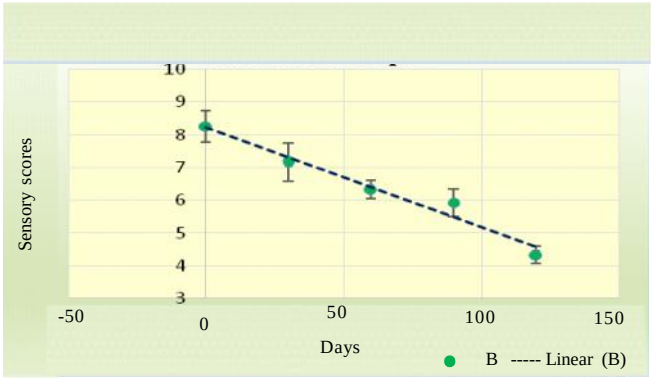

Fig. 4 : Changes in overall acceptability of dry fishes of C sample

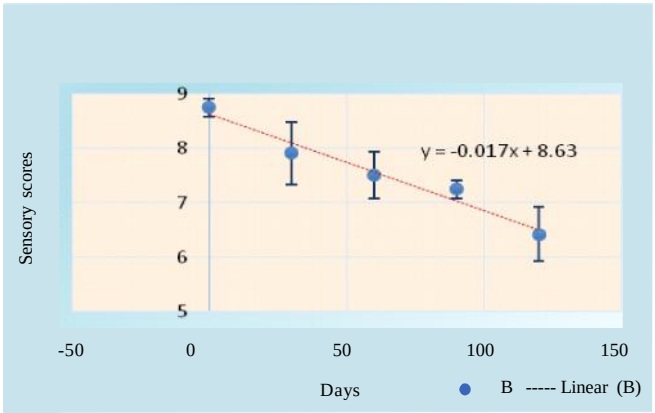

Fig. 5 : Changes in overall acceptability of dry fishes of T_1 sample

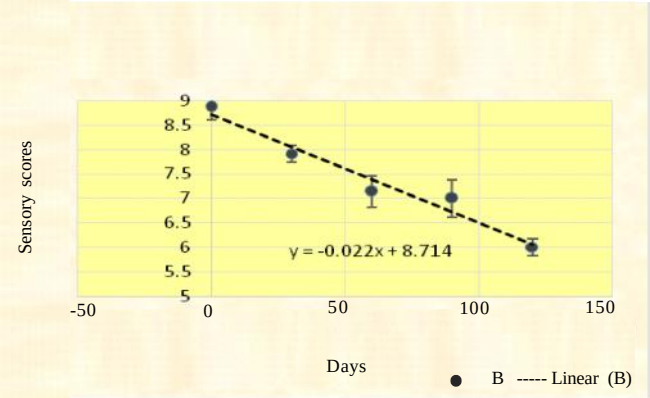

Fig. 6 : Changes in overall acceptability of dry fishes of T_2 sample

experimental findings indicate that the dry salting is better than the brining and use of preservatives enhances quality as well as the shelf-life of the product which shows similarity with the findings of Khuntia *et al.* (1990).

Conclusion :

Dry fish is an important source of animal protein particularly for the poorer section of society. However in India the production process of dry fish is unhygienic which results in inferior quality of the final product. The idea of improving the quality of dry fish by using chemical preservatives was to give extended shelf life to the dry fish which enables entrepreneurs to make it available to the north eastern states of the country with better quality. From the present study it may be concluded that use of permissible food grade chemicals can increase the shelf-life of the dry fish to an extended period which may be beneficial for both producers and consumers.

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